

Classical and Molecular Breeding to Combat PD

Andy Walker, Alan Tenschler, Summaira Riaz, Rachel Graziani, Rong Hu, Kurt Kabica, Geoff Dervishian, Jeremiah Baumgartel, Josh Rubin, Kelly Graves, Adrianna Gozza

David Ramming

Support from CDFA PD/GWSS Board and the Louis P. Martini Endowed Chair Funds



History of Breeding for PD Resistance

- Muscadine grapes have excellent PD resistance, but hard to introgress into *vinifera*
- Bunch grapes in the southeastern US - Mortensen's efforts (Blanc du Bois, BD5-117)... hindered by intense fungal disease pressure and quantitative inheritance of PD resistance

Breeding Objectives

- Breed PD resistant wine grapes through backcross techniques using high quality *V. vinifera* wine grape cultivars and a variety of Xf resistant selections.
- Characterize Xf resistance and wine grape quality traits (color, tannin, ripening dates, flavor, productivity, etc).
- Work with David Ramming to accomplish the same goals with table and raisin grapes.

Backcross Breeding

- Begin with resistance source crossed to *V. vinifera*
- That F1 is 50% *V. vinifera*
- Cross back to *V. vinifera* – 75%
- Cross back to *V. vinifera* – 87.5%
- Cross back to *V. vinifera* – 93.75%
- Cross back to *V. vinifera* – 96.89%
- With marker-aided selection (MAS) have a 3-yr cycle

2007 Crosses

Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.

Resistant Selection	<i>Vinifera</i> Parent of Resistant Selection	<i>Vinifera</i> Cultivars Used in 2007 Crosses	# Seeds Produced
U0501	Syrah	F2-7, F2-35	478
U0502	Chardonnay	F2-7, F2-35	2,769
U0503	Sauvignon blanc	Chardonnay, Palomino, Semillon	126
U0505	Cabernet Sauvignon	Chardonnay, F2-7, LCC, Merlot, Palomino, Petite Sirah	3,229

2007 Crosses

Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 87.5% *V. vinifera* parentage.

Resistant Selection	<i>Vinifera</i> Parent of Resistant Selection	<i>Vinifera</i> Cultivars Used in 2007 Crosses	# Seeds Produced
05310	Alicante Bouschet	Burger, Carignane, LCC	1,666
05312	Cabernet Franc	Zinfandel	194
05317	Tempranillo	Burger, LCC	371
05319	Zinfandel	Cabernet Franc, LCC	144
A81-17	A38-7	Carignane, Grenache noir, LCC	705

2007 Crosses

Monterrey *V. arizonica/candicans* resistance source (F8909-08), and *Run1* and *Vitis* powdery mildew resistance.

Resistant Selection	<i>Vinifera</i> Parent of Resistant Selection	<i>Vinifera</i> Cultivars Used in 2007 Crosses	# Seeds Produced
U0501, U0504 U0502	Syrah Chardonnay	e-series, e78 and e88 allele patterns e-series, e78 and e88 allele patterns	499 837
U0505	Cabernet Sauvignon	e-series, e78 and e88 allele patterns	642
A81-17	A38-7	e-series, e78 allele pattern	603
U0505, A81-17	Cabernet Sauvignon, A38-7	Villard blanc	348

2007 Winegrape Selections

Reference cultivars and select 87.5% *vinifera* progeny with *PdR1*.

Cultivar/ Selection	Parentage	% <i>vin</i>	2007 Bloom Date	Berry Size (g)	Avg Clstr Wt. (g)	Ripening Season	Prod 1=v low 9=vhigh
Cab. Sauv.	Cab. Franc x S. blanc	100	5/20	1.0	168	mid-late	6
Pinot noir	Historic	100	5/7	1.1	259	early	6
U0501-12	A81-138 x Syrah	87.5	5/7	1.0	90	mid-late	4
U0502-01	A81-138 x Chardonnay	87.5	5/1	1.6	128	mid-late	4
U0502-10	A81-138 x Chardonnay	87.5	5/1	1.4	160	v-early	7
Lenoir	<i>V. aestivalis</i> hybrid	<50	5/12	0.8	201	late	7
Midsouth	DGxGalibert 255-5	<50	5/5	2.2	211	mid-late	6

2007 Winegrape Selections

Juice analysis of advanced selections courtesy of ETS Laboratories, St. Helena, CA.

Cultivar or Selection	L-malic acid (g/L)	□B	K (mg/mL)	pH	TA (g/100mL)	YAN (mg/L)	catechin (mg/L)	tannin (mg/L)	Total antho-cyanins (mg/L)
Cab. Sauv.	2.19	24.9	2460	3.65	0.62	227	59	250	404
Pinot noir	2.43	26.5	2190	3.83	0.49	279	321	842	568
U0501-12	4.20	29.4	2900	3.87	0.68	420	88	802	979
U0502-01	2.90	25.9	2530	3.77	0.61	301	91	564	380
U0502-10	4.92	23.7	2220	3.48	0.85	301	87	588	845
Lenoir	4.32	26.9	2920	3.67	0.75	164	195	341	1801
Midsouth	4.60	18.2	2220	3.49	0.81	278	32	230	971

2007 Winegrape Selections

Sensory evaluation of advanced selections with the *PdR1* resistance source.

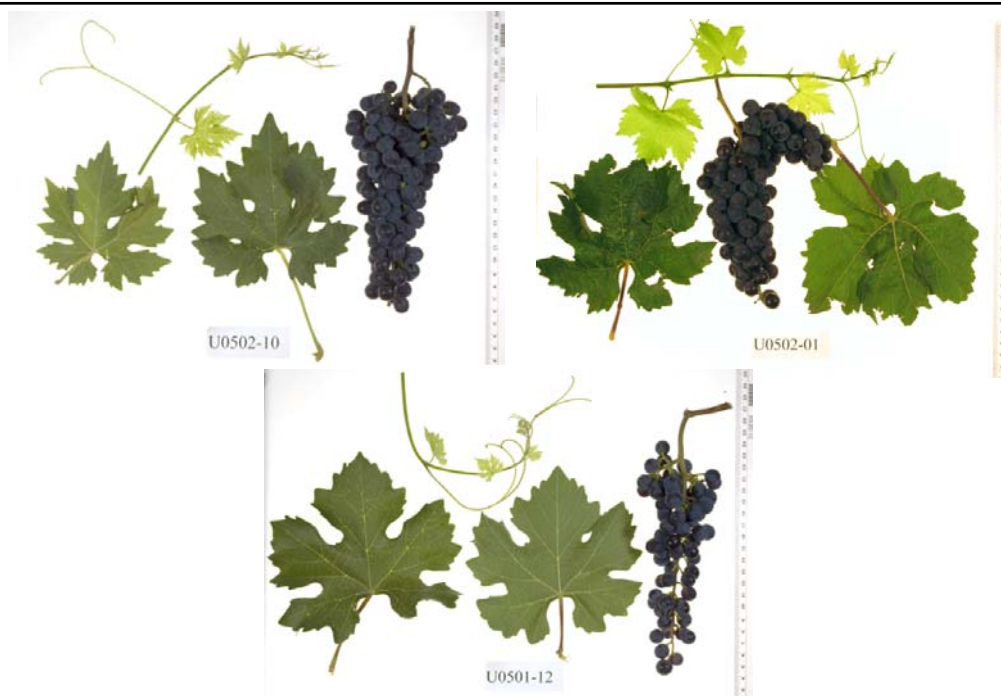
Cultivar or Selection	Juice Hue	Juice Intensity	Juice Flavor	Skin Flavor	Skin Tannin (1=lo, 4=hi)	Seed Color (1=gr 4=brn)	Seed Flavor	Seed Tannin (1=hi, 4=lo)
Cab. Sauv.	pink-brown	lt-med	fruity-CS	fruitjam	2	3	nutty	4
Pinot noir	pink-brown	medium	hay, honey	fruity	1	4	spicy	4
U0501-12	red	med-dark	fruity	fruitjam	2	4	neutral	2
U0502-01	pink-brown	medium	fruity-PN	swt fruit	1	3	spicy	1
U0502-10	pk-red- orng	med-dark	slight veg-dal	mildly fruity	1	4	nutty, spicy	1
Lenoir	red	dark	mildlyfruity	fruity	1	4	nutty	1
Midsouth	red-orange	med-dark	veg-fruity	neutral	1	4	neutral	4

2007 Winegrape Selections

Variety/ Selection	Group Total	Lo Score	Hi Score
U0501-12	33.5	2	4.5
U0502-07	32	2.5	4
Cab Sav	27	2	5
U0502-10	27	1	5
Lenoir	26	2	4.5
U0502-01	24	1.5	4
Pinot noir	20	1	3.5
Midsouth	18.5	1	3

Tasting results of 2007 wines (1= poor to 5 = very good).

There were 9 faculty and staff tasters. See poster for descriptors.



Genetic Objectives

1. Map chromosome 14 around the Xf resistance locus, *PdR1*, in three populations:
04190 (*V. vinifera* F2-7 x F8909-08)
04191 (*V. vinifera* F2-7 x F8909-17)
04373 (*V. vinifera* F2-35 x *V. arizonica* b43-17).
2. Create a BAC (bacterial artificial chromosome) library of the homozygous resistant b43-17 to initiate physical mapping and characterization of *PdR1*

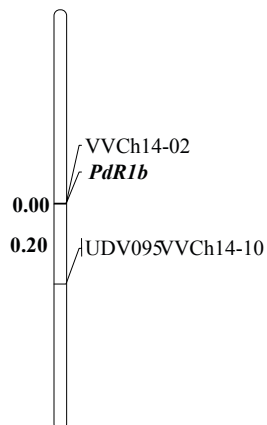
Mapping and Characterizing PD Resistance

- The *PdR1* resistance locus originated from *V. arizonica* b43-17
- All progeny from this plant are resistant to *X. fastidiosa* – the F8909 group (*V. rupestris* x b43-17)
- F8909-08 and F8909-17 have been used in breeding and mapping Xf resistance
- D8909-15 x F8909-17 = 9621 population

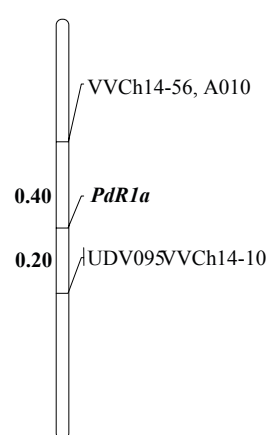
Mapping and Characterizing PD Resistance

- Fine scale mapping has positioned *PdR1* to within 1cM of markers on LG 14
- We have mapped *PdR1* from F8909-17 and F8909-08 and they map differently and represent either chromatid of the *PdR1* locus – *PdR1a* and *PdR1b* from b43-17

**F8909-08
04190 population**



**F8909-17
9621 population**

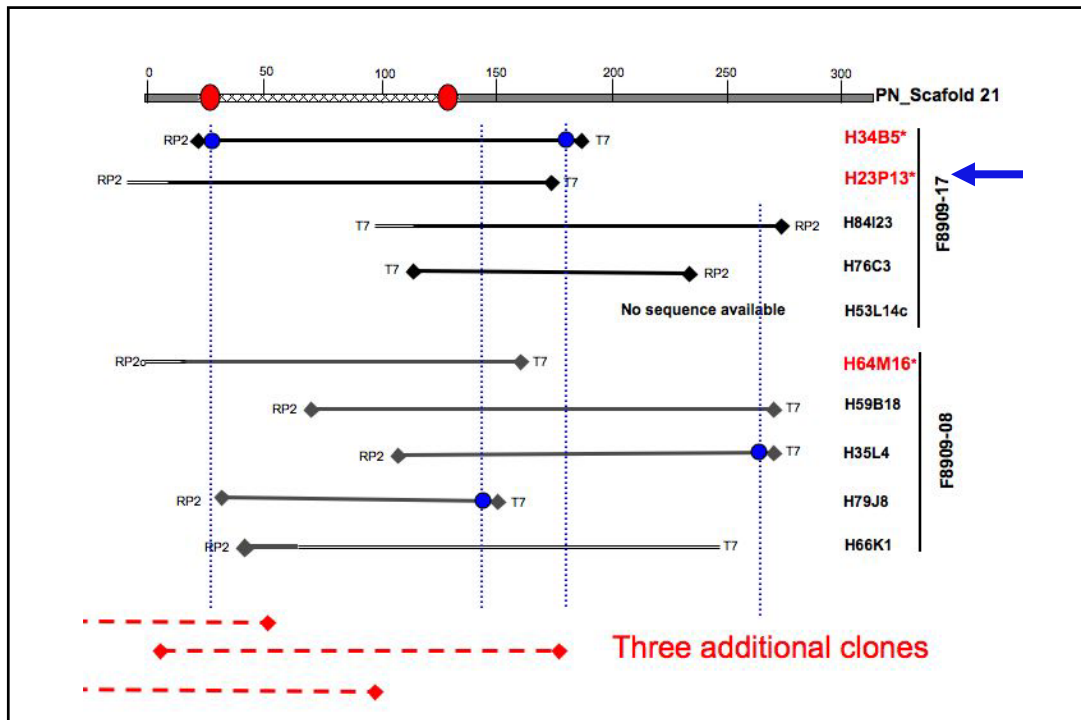


Physical Mapping Progress

- Have constructed BAC libraries of b43-17 with two restriction enzymes
- The *Hind* III library consists of 34,560 clones with an average insert size of 140 Kb and a 12X coverage
- The *Mbo* I library consists of 23,040 clones with an average insert size of 130 Kb and an 8X coverage

Physical Mapping Progress

- Two flanking markers were used to screen the libraries – VVCh14-10 and VVCh14-56
- Sequence information from the Pinot noir genome and BAC end sequence information was then used to align the BAC pieces
- Compared sequences of the two tightly linked markers (VVCh4-10 and VVCh4-56) to the PN sequence and located a 109 Kb region on scaffold 21 of Chromosome 14



Sources of Xf resistance

- Comparisons of the PN sequence based on our *PdR1* flanking markers identified a 109 Kb region with 13 genes— cell wall metabolism genes, a protein kinase, and 6 genes of unknown function
- Gene characterization and verification will begin once sequencing is completed
- We are preparing to have a second clone representing *PdR1b* sequenced

Other Sources of Xf resistance

- b42-26 – (*V. arizonica/girdiana*) from Loreto, BC its resistance is inherited as a quantitative trait
- We are mapping in a BC1 generation (D8909-15 (*rupestris* x b42-26) x *vinifera*) x *vinifera* for quantitative trait loci (QTL)
- Some promising QTL have been mapped, but more resistance phenotype and markers are needed.

Sources of Xf resistance

- b40-14 – *V. arizonica* from Chihuahua, its Xf resistance also appears to be inherited as a single dominant gene (all *rupestris* and *vinifera* F1s are resistant)
- 2007 crosses to create BC1 mapping population
- Create framework map to locate Xf resistance locus

Sources of Xf resistance

- Markers linked to *PdR1* from b43-17 have different alleles in b40-14 and b42-26
- Many more *V. arizonica* and *V. girdiana* forms have been collected for testing

Sources of Xf resistance

- In conjunction with David Ramming we are using BD5-117 (Daytona x Stover) for table grape breeding. Its resistance is reported to be inherited as a multi-gene trait.
- *V. vinifera* C33-30 x BD5-117 (Daytona x Stover) 150 progeny are being screened for QTL mapping purposes.

Wine Evaluations

- Need to evaluate 100s of resistant selections per year.
- What quality parameters will be useful in predicting wine quality at the 2L, 20L and 2,000L scale? - Kelly Graves
- Diglucoside anthocyanins? - Adrianna Gozza

Breeding Objectives

- Develop large seedling populations at the 94% and 97% *vinifera* level in many diverse and high quality *vinifera* winegrape backgrounds
- Intercross advanced high quality selections with Xf resistance from multiple sources
- Develop genetic markers for fruit and wine quality

Thanks!

